Shiga toxin-producing Escherichia coli (STEC) isolated from fecal samples of African dromedary camels

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Shiga toxin-producing *Escherichia coli* (STEC) isolated from fecal samples of African dromedary camels

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ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) cause gastrointestinal illnesses including non-bloody or bloody diarrhoea, haemorrhagic colitis (HC), and the haemolytic uremic syndrome (HUS). To investigate the occurrence of STEC among grazing dromedaries from Kenya, *E. coli* isolated from fecal matter collected from 163 dromedaries on a large ranch were screened for the presence of *stx1* and *stx2*. STEC strains were isolated and serotyped. Isolates were subjected to PCR for the subtyping of *stx* genes and for the detection of *eae* and *ehx*. In addition, whole genome sequencing (WGS) was carried out to detect further virulence genes and to determine the multilocus sequence types (MLST). Antimicrobial resistance profiles were determined by disk diffusion.

STEC was isolated from 20 (12.3%) of the fecal samples. Thereof, nine (45%) isolates were STEC O156:H25, three (15%) isolates typed STEC O43:H2. The remaining isolates occurred as single serotypes or were O non-typeable. Eleven (55%) of the isolates harboured *stx2a*, nine (45%) *eae*, and 14 (70%) *ehx*, respectively. WGS revealed the presence of *iss* in 16 (80%), *subAB* in four (20%) and *astA* in two (10%) of the isolates, Furthermore, *espA*, *tccP*, *nleA*, *nleB*, *tccP*, and *tir* were found exclusively among STEC O156:H25.

Eleven different sequence types (ST) were detected. The most prominent was ST300/ST5343, which comprised STEC O156:H25. All STEC isolates were pan susceptible to a panel of 16 antimicrobial agents. Overall, the results indicate that dromedary camels in Kenya may be reservoirs of STEC, including serotypes possessing virulence markers associated to disease in humans, such as STEC O156:H25. STEC in camels may represent a health hazard for humans with close contact to camels or to consumers of camel derived foodstuffs, such as unpasteurised camel milk.

1. Introduction

In Eastern Africa, the one-humped Arabian camel (*Camelus dromedarius*) is an important livestock species particularly well adapted to semi-arid and arid environments [1]. In Kenya, the husbandry of semi-domesticated dromedaries is a traditional livelihood of the nomadic pastoralists, with milk constituting an important nutrient source and income asset [2].

Kenya as a country relies heavily on wildlife tourism [3]. In the Laikipia county in central Kenya which is known for its remarkable abundance of wildlife, livestock husbandry and tourism are alternatives to land cultivation [4,5]. Dromedary camel herds have become established both in pastoral communities and on large ranches where they are used for wildlife safaris and to produce milk for consumption and trade [1].

Camels in sub-saharan Africa reportedly represent a reservoir for a variety of potentially zoonotic diseases and pathogens, including *Coxiella burnetii* [6], *Brucella* spp. [7], Hepatitis E Virus [8], and Middle East respiratory syndrome coronavirus [9]. By contrast, Shiga toxin-producing *Escherichia coli* (STEC), which may cause gastrointestinal illnesses including non-bloody or bloody diarrhoea, haemorrhagic colitis (HC), and the haemolytic uremic syndrome (HUS) [10], has rarely been described in camels. STEC causes an estimated 2,801,000 acute illnesses annually and poses a significant health burden worldwide [11,12].

The distinguishing feature of STEC is the presence of one or more

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Table 1
Examples of healthy (semi)-domestic animals as hosts of Shiga toxin-producing *Escherichia coli* (STEC) serotypes associated with disease in humans.

Animal	STEC serotype(s)	Reference(s)
Camels	O157:H7, O156:H25	[21], this study
Cattle	O157:H7, O156:H25, O8:H19, O26:H2, O26:H8, O26:H11, O26:H19, O26:H21, O45:H2, O103:H2, O103:H21, O121:H8, O145:H2, O145:H8, O145:H28, O146:H21	[29,36–40]
Chicken	O157:H7	[36,37]
Goats	O146:H21, O82:H8, O128:H2	[40–42]
Sheep	O157:H7, O26:H11, O146:H21, O128:H2	[37,40,41,43]
Swine	O157:H7	[36,37]
Turkeys	O157:H7	[36]

Shiga toxin (*stx*) genes which include two major forms, *stx1* and *stx2* and subtypes thereof [13]. Other major virulence genes that influence the pathogenic potential of STEC include the intimin gene *eae*, and the enterohemolysin gene *ehx* [14,15]. Additional virulence factors such as *espA*, *nleA*, *iss* or *subAB* are also frequently associated with disease in humans [16,17].

STEC O157:H7 is the serotype most frequently associated with severe clinical outcomes, nonetheless, non-O157 STEC serogroups, including O26, O103, O111, and O145, are also recognized for their pathogenic potential [18].

Wild and domestic animals, including cattle, sheep, goats and deer represent the most important reservoirs of STEC [12]. Examples of healthy domestic animals that are known to be hosts of human pathogenic STEC serotypes are given in Table 1. The consumption of contaminated water, meat, raw milk, raw milk products and produce, and the direct contact with farm animals have been identified as important routes of transmission [12,19]. A better understanding of the occurrence of STEC among animal reservoirs is essential to provide a framework for developing approaches to reduce the burden of STEC related disease worldwide.

So far, *E. coli* O157:H7 and STEC O157:H7 was isolated from meat and feces of camels in Iran, respectively [20,21]. By contrast, several studies have reported the absence of STEC in camels in the United Arab Emirats and Eastern Africa [22–24].

This study aimed to update the current understanding on the occurrence of STEC in camels in Eastern Africa by screening fecal samples of a herd of healthy animals kept under extensive husbandry systems on a ranch in Laikipia, Kenya, and to characterize all STEC strains with regard to their serotypes and their virulence potential.

2. Material and methods

2.1. Sample collection

Fecal samples of 163 healthy dromedary camels (117 females, 45 males and one of unknown gender) located in a ranch in the northern Laikipia county in Kenya were collected during January and February 2017. The camels were extensively kept, they shared their pasture with game and cattle. Fecal matter was collected by rectal palpation using one glove per animal. Samples were placed in sterile containers, cooled for transport and frozen at −80 °C.

The sample collection was performed in strict accordance with the Kenyan legislation for animal experimentation and were approved by the institutional animal care and use committee (IACUC reference number 2014.08). Since 1993, the International Livestock Research Institute (ILRI) in Kenya has complied voluntarily with the United Kingdom's Animals (Scientific Procedures) Act 1986 that contains guidelines and codes of practice for the housing and care of animals used in scientific procedures.

2.2. Screening for stx by PCR

Each fecal sample was enriched at a 1:10 ratio in Enterobacteriaceae enrichment (EE) broth (Becton, Dickinson, Heidelberg, Germany) for 24 h at 37 °C. One loopful of each of the enrichment cultures was cultured on sheep blood agar (Difco™ Columbia Blood Agar Base EH; Becton Dickinson AG, Allschwil, Switzerland) using the streak plate technique. The resulting colonies were washed off with 2 ml 0.85% NaCl and DNA was extracted by a standard lysis protocol. Screening for *stx1 and stx2* genes was performed by real-time PCR (LightCycler R 2.0 Instrument, Roche Diagnostics Corporation, Indianapolis, IN, USA) using the QuantiFast Multiplex PCR Kit (Qiagen, Hombrechtikon, Switzerland) according to the guidelines of the European Union Reference Laboratory (EURL) [25].

2.3. Recovery of STEC

In the event of a *stx* positive PCR result, one loopful each of the enrichment broth was streaked onto on sheep blood agar (Difco™ Columbia Blood Agar Base EH; Becton Dickinson AG, Allschwil, Switzerland) and onto RAPID'*E. coli* Agar (BioRad, Basel, Switzerland) to obtain single colonies of *E. coli*.

From each plate, individual colonies were picked and suspended in 0.5 ml NaCl 0.85%. Isolates that were confirmed to possess *stx* (*stx1*and/or *stx2*) by real-time PCR were subcultured on sheep blood agar and the presence of *stx* was confirmed using the Assurance GDS® for Shiga Toxin Genes (Bio Control Systems, Bellevue, WA, USA). From plates yielding more than one *stx* positive colony, one isolate was randomly chosen for subsequent characterization.

2.4. Serotyping and molecular typing of stx, eae and ehx genes

Isolates were serotyped using the *E. coli* SeroGenoTyping AS-1 Kit (Alere Technologies, Jena, Germany). The determination of *stx1* subtypes (*stx1a, stx1c*, *stx1d*) and *stx2* subtypes (*stx2a*, *stx2b*, *stx2c*, *stx2d*, *stx2e*, *stx2g*) was performed by conventional PCR amplification [13]. Screening for *eae, and ehx* was performed by real-time PCR according to the guidelines of the EURL [26].

2.5. Whole genome sequencing

Genomic DNA libraries were prepared using the Nextera™ DNA Flex Library Preparation Kit following the manufacturer's protocol (Illumina, San Diego, CA, USA). Libraries were loaded on a 300-cycle, 2 × 150 bp paired-end read cartridge (Illumina MiniSeq High Output Reagent Kit) and run on a MiniSeq Illumina sequencer (Illumina). The Spades assembler v3.12 [27] was used to assemble the reads with using the option "—careful". The spades output was filtered for size > 1000 bp and coverage > 25-fold resulting in the final assemblies that were submitted to NCBI GenBank.

The sequence reads were used to run analysis in the Advanced Research Infrastructure for Experimentation in genomics (ARIES) public Galaxy server (https://w3.iss.it/site/aries/), i.e. quality control, assembly, virulence gene analysis, Shiga-toxin analysis, and serotyping.

To assess the genetic relatedness of the isolates, core genome multilocus sequence typing (cgMLST) was carried out using Ridom SeqSphere Software (version 4.1.9, available at http://www.ridom.de/seqsphere/cgmlst/). The MLST schemes "Warwick" and "Pasteur" were used for MLST-typing, and the Enterobase *Escherichia/Shigella* v1 scheme (http://enterobase.warwick.ac.uk) for cgMLST typing. Assembled genomes were blasted against the reference database using standard settings and the option "ignore missing values pairwise" and "Discard genomes with > 3% missing genes" were used for typing. Trees were visualised using the minimal spanning tree option in seqsphere.

2.6. Antibiotic resistance profiles

Antimicrobial susceptibility testing was performed using the disk-diffusion method and the antibiotics ampicillin (AM), amoxicillin-clavulanic acid (AMC), cefazolin (CZ), cefotaxime (CTX), cefepime (FEP), nalidixic acid (NA), ciprofloxacin (CIP), gentamicin (GM), kanamycin (K), streptomycin (S), sulfamethoxazole/trimethoprim (SXT), fosfomycin (FOS), azithromycin (AZM), nitrofurantoin (F/M), chloramphenicol (C) and tetracycline (T) (Becton Dickinson, Heidelberg, Germany). Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) performance standards [28].

3. Results and discussion

In sub-Saharan Africa, little information is available on the epidemiology of STEC in humans, food and animals, and current knowledge of STEC sources needs to be improved [12]. Data on the occurrence and the characteristics of STEC in African camels is limited. This study provides evidence for the fecal carriage of STEC among dromedary camels located on a ranch in Laikipia Kenya. The restriction to one farm in Kenya is a limitation of this study and may have led to bias associated with camels from one herd only. In order to obtain a wider picture, analyses of feces from camels on other farms and other regions in Kenya would be required. For this investigation, a total of 163 fecal swabs were obtained. Thereof, 53 (32.5%) tested positive for *stx* by real-time PCR. STEC was recovered from 20 of the *stx* positive samples, amounting to 12.3% of all fecal samples. Overall, fecal carriage of STEC was confirmed for 14.5% (17 of a total of 117) of the female camels and 6.7% (3 of 45) of the male camels (Table 2).

The serotypes and virulence genotypes of the STEC isolates are summarized in Table 2. The most frequent serotypes included nine (45% of the isolates) STEC O156:H25 isolates and three (15%) O43:H2 isolates. The remaining strains occurred as single serotypes or were O non-typeable (Table 2). STEC O156:H25 has been described as a persistent colonizer of the bovine gut [29] and has also been linked to human infection in Germany and Switzerland [30,31] (Table 1). By contrast, STEC O43:H2 is a rare serotype, but has been detected in beef in West Africa [32].

Further, various *stx* subtypes were detected among the isolates, including *stx2a* which is associated with severe disease in STEC infected humans [33]. The *stx2a* gene was found in a total of 11 (55%) of the isolates, including the nine STEC O156:H25 strains, and the STEC O8:H49 and STEC Ont:H7 isolates, respectively (Table 2). The intimin gene *eae* was detected in nine (45%) isolates, all belonging to serotype O156:H25 (Table 2). While *eae* is one of the most prominent virulence factors contributing to pathogenesis, many STEC feature other virulence genes that may enhance their pathogenic potential, such as enterohaemorrhagic *E. coli* haemolysin *ehx* [15,30]. In this study, *ehx* was detected by PCR in fourteen (70%) of the isolates (Table 2).

Furthermore, we used WGS to provide additional information on the virulence of the analysed STEC. Table 2 shows the presence or absence of a selection of virulence genes that predict severe disease in humans, including *espA*, *tccP*, *nleA*, *nleB* and *tir* (de Boer et al., [16]). Other virulence factors included in Table 2 are *iss*, which encodes an increased serum survival protein and is associated with non-bloody diarrhoea as well as extra-intestinal pathogenic infections [34], *subAB*, which is an emerging pathogenic factor among *eae* negative human pathogenic STEC isolates [17], and *astA* encoding a heat-stable enterotoxin EAST1 that is found mostly in enterohaemorrhagic and enteroaggregative *E. coli* [35]. The complete ARIES analysis reports on the virulotypes of the 20 sequenced strains are available upon request.

Taken together, *espA*, *tccP*, *nleA*, *nleB* and *tir* were detected exclusively among the nine STEC O156:H25 isolates, while *iss* was identified in 16 (80%), and *subAB* in four (20%) of the isolates, respectively (Table 2). A minority of two strains (10%) possessed *astA* (Table 2). Although all STEC can conceivably cause diarrhoea, the risk and severity of infection is linked to the presence of virulence factors, in particular *stx2a* and *eae* [12]. Our results show that although 45% of the STEC isolated from camel feces are not highly pathogenic according to their virulence factor profiles, the majority (55%) is associated with *stx2a,* and STEC O156:H25 isolates in particular, exhibit a virulotype that suggests a potential health risk.

Using the Pasteur scheme, four different sequence types (ST) were identified among 14 isolates (Table 2). Using the Warwick scheme, 11 different STs were identified (Table 2). Of the nine STEC O156:H25 isolates, eight grouped in the sequence type ST300 and ST5334, which

Table 2
Characteristics of Shiga toxin-producing *Escherichia coli* (STEC) isolated from feces of camels from Kenya.

Strain ID	Host gender	Serotype	Virulence genes										MLST		GenBank accession no.
			stx subtype	*eae*	*ehx*	*astA*	*espA*	*iss*	*nleA*	*nleB*	*tccP*	*tir*	*subAB*	ST Warwick	ST Pasteur	
C59	F	O8:H19	*stx1a*	−	+	−	−	+	−	−	−	−	−	201	294	RJDD00000000
C102	F	O8:H49	*stx2a*	−	+	−	−	+	−	−	−	−	−	111	nd	RJDT00000000
C139	F	O43:H2	*stx1c*	−	−	−	−	+	−	−	−	−	−	937	363	RJDN00000000
C119	F	O43:H2	*stx1a*	−	−	−	−	+	−	−	−	−	+	6275	363	RJDS00000000
C160	F	O43:H2	*stx1c*	−	−	−	−	+	−	−	−	−	+	6275	363	RJDK00000000
C34	F	O44:H18	*stx1a, stx2c*	−	+	−	−	+	−	−	−	−	+	6507	nd	RJDG00000000
C21	F	O117:H12	*stx1a*	−	−	−	−	+	−	−	−	−	−	101	nd	RJDJ00000000
C22	F	O150:H8	*stx1c*	−	+	+	−	−	−	−	−	−	+	906	303	RJDI00000000
C12	F	O156:H25	*stx2a*	+	+	−	+	+	+	+	+	−	−	300	591	RJDR00000000
C15	F	O156:H25	*stx2a*	+	+	−	+	+	+	+	−	+	−	300	591	RJDM00000000
C16	F	O156:H25	*stx2a*	+	+	−	−	−	−	−	−	−	−	300	591	RJDL00000000
C25	F	O156:H25	*stx2a*	+	+	−	+	+	+	+	+	+	−	300	591	RJDH00000000
C43	F	O156:H25	*stx2a*	+	+	−	+	−	+	+	−	+	−	300	591	RJDF00000000
C62	F	O156:H25	*stx2a*	+	+	−	+	+	+	+	−	+	−	300	591	RJDC00000000
C85	F	O156:H25	*stx2a*	+	+	−	+	+	+	+	−	+	−	300	591	RJDB00000000
C129	M	O156:H25	*stx2a*	+	+	−	+	+	+	+	−	+	−	300	591	RJDP00000000
C101	M	O156:H25	*stx2a*	+	+	+	+	+	+	+	+	+	−	5343	591	RJDU00000000
C127	F	Ont:H7	*stx1a, stx2a*	−	+	−	−	−	−	−	−	−	−	1308	nd	RJDQ00000000
C56	M	Ont:H20	*stx1c*	−	−	−	−	+	−	−	−	−	−	5759	nd	RJDE00000000
C131	F	Ont:H20	*stx1c*	−	−	−	−	+	−	−	−	−	−	5759	nd	RJDO00000000

astA, gene encoding enteroaggregative heat-stable toxin; *eae*, intimin gene; *ehx,* enterohemolysin gene; *espA*, gene encoding LEE-associated secreted translocator protein; F, female; *iss*, gene encoding increased serum survival protein; M, male; MLST, multilocus sequence type; nd, not defined; *nle*, genes encoding non-LEE-encoded factors; ST, sequence type; *subAB*, subtilase cytotoxin gene; *tccP*, gene encoding Tir cytoskeleton coupling protein; *tir*, gene encoding translocated intimin receptor; +, the gene is present; −, the gene is absent.

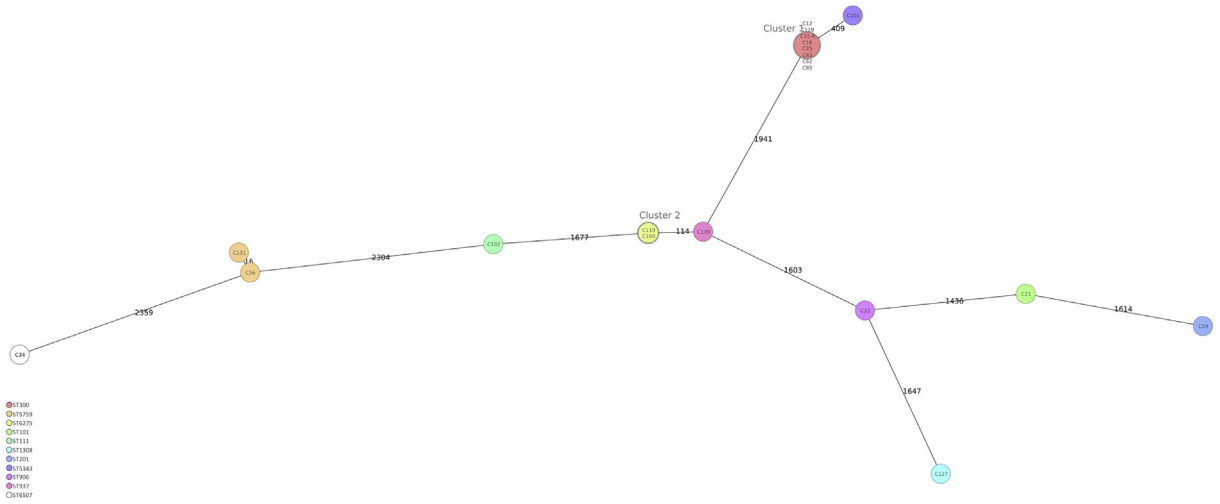

Fig. 1. Phylogenetic relationship of 20 Shiga toxin-producing *Escherichia coli* (STEC) isolated from camel feces based on their multilocus sequence type (MLST) allelic profiles. The minimum spanning tree was generated using SeqSphere (Ridom GmbH). Numbers in the lines indicate the number of allele differences between isolates. The size of each circle reflects the number of isolates allocated to an individual sequence type (ST). The colours of the circles represent STs according to the Warwick scheme (http://enterobase.warwick.ac.uk). Strain IDs are indicated in the circles.

differ by one nucleotide in the *gyrB* gene, indicating close phylogenetic relationship of the isolates. Similarly, the three STEC O43:H2 isolates were assigned to ST937 and ST6275 which differ by one nucleotide in *fumC*. Two STEC Ont:H20 possessed ST5759. All other isolates belonged to singly occurring STs (Table 2). Accordingly, cgMLST using Ridom SeqSphere+ software divided the strains into distinct clusters that comprised the STEC O156:H25 isolates, the STEC O43:H2 isolates, and the Ont:H20 isolates, respectively, while the remaining strains did not reveal close phylogenetic relationship (Fig. 1).

Finally, all STEC isolates remained fully susceptible to all antimicrobials tested (data not shown).

4. Conclusions

The data from our study suggest that dromedary camels are a reservoir of human pathogenic STEC. While none of the fecal samples contained STEC O157:H7 or other non-O157 STEC serogroups frequently associated with severe clinical outcomes such as bloody diarrhoea, HC, or HUS, the majority of the isolates harboured *stx2a*, and the STEC O156:H25 isolates harboured *eae*, *ehx*, and other virulence factors often associated with disease in humans. Their occurrence in fecal samples from camels may represent a threat to humans, especially to those who live in long-term close contact with camels and consume raw camel milk.

Nucleotide sequences

The nucleotide sequences have been deposited at GenBank under accession nos. RJDB00000000-RJBU00000000.

Disclosures

The authors report no conflict of interest. All authors have read and approved the final article.

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